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Testing for Transferred Immunity of a Universal Influenza Vaccine in Pigs

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by

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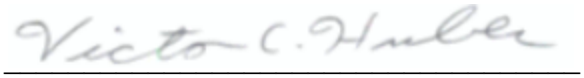

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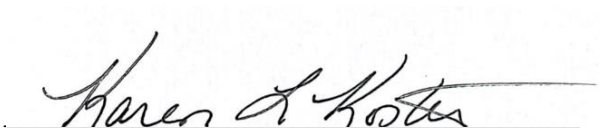

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ABSTRACT

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Influenza causes high numbers of illnesses and deaths annually (CDC, 2020). Influenza vaccines prevent these complications by reducing the risk of flu illness between 40-60% (CDC, 2021). However, no vaccination exists for infants under six months old so other methods, such as passive immunity, must be explored. To determine how infants can be protected, we tested the passive transfer of a universal influenza vaccine using a pig model and researched the mechanism of transfer. Four pregnant pigs were vaccinated one time with PBS and one time with HA-129 vaccine and four were vaccinated twice with the HA-129 vaccine. After farrowing, piglets were challenged with influenza virus and nasal swabs were taken and analyzed to determine whether piglets were infected. We tested the hypothesis that if vaccinated mother pigs transfer high levels of antibodies to the newborn, then antibodies will protect influenza-challenged offspring from the virus. Our results found higher mean viral titer values for the PBS vaccinated group indicating higher levels of infection. Despite error within research protocols, such as the lack of a true PBS group, we can predict that influenza vaccination of mothers may lead to the protection of piglets. Our research forms a basis for vaccination of pregnant mothers to protect the immunocompromised mother and vulnerable newborn.

Keywords: Influenza, Maternal, Transfer, Pigs, Immunity, Vaccine, Universal

TABLE OF CONTENTS

CHAPTER ONE

Introduction

The History of Influenza

The words epidemic and pandemic have been a central theme of discussion throughout the past two years. An epidemic is an increase in the prevalence of disease above the expected number in the population, while a pandemic is an epidemic that affects many people and spreads over several continents (CDC, 2012). These classifications are not new, as they have been applied to the influenza virus for at least three hundred years (Potter, 2001). Epidemics and pandemics occur due to an increase in virulence or ability to cause disease, a lack of natural immune responses to the antigen, and increased transmissibility (CDC, 2012). Specific genetic mutations affecting the viral life cycle are called virulence determinants, which contribute to increased severity and transmissibility in highly pathogenic phenotypes of the influenza virus (Tscherne, 2011). These mutations include factors increasing evasion of innate and adaptive immune response; changing genome replication, transcription, and translation; and altering virus binding, entry, assembly, and release (Tscherne, 2011). A major example of a virulence determinant is hemagglutinin changes that rapidly increase viral transmission (Tscherne, 2011). Transmission of the influenza virus is an ongoing struggle that decreases global health annually due to high mutation rates and increased antigenic variation, which decreases the natural ability of the immune system to respond to the virus. These characteristics create difficulties in establishing a protective vaccine (Abbas, 2020). A lack of herd immunity, yearly vaccination requirements, and insufficient natural

responses allow for the high transmissibility of influenza between hosts, resulting in the high infection rates seen each year.

The issues of antigenic variation and a lack of herd immunity cause the world to be impacted by influenza epidemics every year and pandemics every ten to fifty years, typically during the winter months (Potter, 2001). Influenza epidemics have been recorded throughout history with the first possible Greek epidemic occurring in 412 BCE (Ghendon, 1994 & Potter, 2001). The first report of an epidemic with clear symptoms aligning with influenza occurred in 1173 followed by several reports of influenza outbreaks in the 14th and 15th centuries which are considered indisputable influenza epidemics (Ghendon, 1994). These early epidemics were followed by records in America and Europe during the 17th century, accounts in the 18th and 19th centuries, and current infections continuing today (Potter, 2001). Epidemics are generally limited to specific geographic areas and cause fewer worldwide death rates; meanwhile, pandemics cause high death rates and spread to much of the world's population. Pandemics caused by the influenza virus have negatively impacted population health throughout history. This impact can be seen by recognizing the most notable pandemics. During the outbreak of 1510 in Africa and Europe, people experienced cough, fever, and constriction (Morens, 2010). This was the first recognition of pandemic influenza with a high attack rate and few deaths, but the effects of influenza were much more devastating during the highly transmissible and deadly 1580 pandemic beginning in Asia and spreading globally with a 95% infection rate in Europe and 8,000 deaths in Rome (Ghendon, 1994 & Potter, 2001). Subsequent pandemics with high global transmission rates were seen during the 1729 pandemic, which imposed high death rates globally with increased severity in later waves

of infection; the 1781 pandemic with high morbidity and mortality rates in young adults; and the 1830 pandemic characterized by low death rates (Potter, 2001). During 1918-19, a pandemic that was first classified as bacterial emerged; however, in1933, it was characterized as the first influenza virus originating from swine and the variant was classified as the HswN1 subtype (Barberis, 2016; Ghendon, 1994). The 1918 pandemic, also known as the Spanish Flu, is characterized as one of the most destructive pandemics causing forty to fifty million deaths with infection of half of the world's population (Ghendon, 1994; Potter, 2001). The severity caused public events to cease, schools and churches to close, and masking to be implemented (Ghendon, 1994). The 1957-1958 pandemic of the H2N2 virus emerged from avian origin and infected 40% to 50% of the world's population and killed about one million (Tscherne, 2011). Another avian virus (H3N2) was prominent in the 1968 pandemic, killing one million people worldwide. Lastly, the 2009 pandemic of H1N1 originated in swine and caused 60.8 million cases, 274,304 hospitalizations, and 12,469 deaths in the United States and 0.001% to 0.007% of the world's population died due to infection (CDC, 2019; Potter, 2001). This pandemic increased the urgency to implement effective pandemic planning, including substantial efforts to develop universal vaccines to decrease pandemic death rates (Morens, 2010).

Prevalence and Importance

The influenza virus is a leading cause of death and severe disease annually (Smith, 2018). Although vaccines and antiviral therapies have been developed, the evolutionary evasion of the immune response continues to cause pandemics (Smith, 2018). The World Health Organization found three to five million cases of severe illness occur and up to 650,000 people die annually due to respiratory impacts of influenza

(WHO, 2018; Paget, 2019). Of the 650,000 deaths, 99,000 - 200,000 deaths are associated with direct lower respiratory infections due to influenza and 292,000 - 518,000 deaths are influenza-associated; these associations include secondary bacterial infections or poor outcomes of pre-existing disease (Paget, 2019). Annually, the virus accounts for about two percent of all respiratory deaths globally (Paget, 2019).

The number of respiratory deaths annually varies based on the global region (Paget, 2019). North and South America experience the highest rates of death due to influenza with 6.2 deaths per 100,000 people (Paget, 2019). South-East Asia experiences 5.8 deaths, Africa experiences 5.6 deaths, Europe experiences 5.3 deaths, and the Eastern Mediterranean experiences 4.5 deaths per 100,000 people (Paget, 2019). These differences are due to variable access to healthcare, regional economic profit, varying developmental stages of areas, and different levels of crowding. Within global boundaries, countries experience vast deviations from the average death rates (Cozza, 2021). This can be seen as Mexico and Canada experience death rates of 3.6 to 5.2 deaths per 100,000 people while Chile, Argentina, Bolivia, and Peru experience death rates of 5.3 to 16.5 per 100,000 people compared to the mean rate of 6.2 deaths per 100,000 in the Americas (Cozza, 2021). Regional differences within a country also affect influenza outcomes. This can be seen in India as of those hospitalized due to severe respiratory infection 36% died in Jodhpur Rajasthan, 7% died in Kerala, 25 % died in Saurashtra, and 7 % died in Andhra Pradesh (Fischer, 2014). Based on this evidence, geographic location contributes to variances in influenza morbidities and mortalities.

High mortality and morbidity rates from influenza have not only negatively impacted the globe, but the illness has had devastating impacts on the United States

(Smith, 2018). From 2010 to 2020, influenza caused between nine million and forty-one million illnesses, 140,000-710,000 hospitalizations, and 12,000-52,000 deaths each year (CDC, 2020). The high transmission, morbidity, and mortality rates due to influenza on the local, national, and international levels, despite having access to current vaccines, portray the importance of continuing research to increase the effectiveness of these protective measures.

The high rates of death and illness create immense stress on healthcare infrastructure which can overwhelm public health and healthcare delivery systems. The systems experience stress due to a lack of treatments, equipment, healthcare resources, healthcare facilities, and personnel (Levin, 2007). Disasters from illness are especially devastating because every community is impacted, so there is a lack of personnel and spare medical equipment that can be mobilized for assistance (Levin, 2007). For example, New York city predicted that if a pandemic were to occur in 2007, 67% of intensive care unit beds would be filled in the first wave (Levin, 2007). The large number of infected people in one area increases pandemic severity because it serves as a tool of transmission, causing an increase in spread to healthcare professionals and visitors (Levin, 2007). Influenza also places a large financial burden on the healthcare system and government. This burden includes $3.2 billion in direct medical costs and $8.0 billion in indirect costs (Putri, 2018). A combination of death tolls, severe illnesses, transmission to vulnerable groups, and economic burden establishes influenza as an important issue to be researched and mitigated.

The extreme outcome of influenza infection is death, but influenza also causes mild to severe symptoms (WHO, 2018). These symptoms include fever, fatigue, dry

cough, headache and migraines, joint pain, muscle pain, body aches, vomiting, diarrhea, severe malaise, runny or stuffy nose, and sore throat that last about two weeks for mild cases (WHO, 2018; CDC, 2021). Moderate complications include ear and sinus infections (CDC, 2021). Cases that persist or have more severe symptoms may require hospitalization. Hospitalization is often due to secondary respiratory diseases, such as pneumonia (CDC, 2021). Other severe complications caused by influenza are myocarditis, encephalitis, myositis, sepsis, rhabdomyolysis, extreme inflammatory response, and organ failure (CDC, 2021).

Symptoms and respiratory diseases cause death and severe illness, but certain populations are affected disproportionately. Those experiencing chronic disease, weakened immune systems, pregnancy, young age, and old age are more likely to develop severe disease or complications due to influenza (WHO, 2018). Influenza is likely to exacerbate symptoms for those with previous medical conditions or chronic disease, resulting in poorer health outcomes (CDC, 2021; WHO, 2018). Another vulnerable population includes those that are pregnant (WHO, 2018). During the 1918 pandemic, mortalities of pregnant women were 5.3 to 5.7 per thousand people compared to 4.9 per thousand in the entire women's population (Reid, 2005). Further, pregnant women were two times more likely to develop pneumonic complications, and with these complications, pregnant women were two times more likely to die than non-pregnant women (Reid, 2005). The fetus was also affected during 1918-19 as the miscarriages linked to influenza rose to a height of 1.6 per thousand (Reid, 2005). In the United States, fetal loss occurred in 26% of uncomplicated influenza cases and 52% of severe cases among pregnant women (Reid, 2005). Influenza continues to pose a threat after birth. In

1918-19 at Derbyshire, 3.67% of influenza deaths were infants (Reid, 2005). This remains a current issue as there were 144 pediatric deaths from 2018 to 2019 and 199 pediatric deaths from 2019 to 2020 due to influenza in the US (CDC, 2021). Those 65 and older are also disproportionately affected, accounting for 67% of global influenza-associated deaths (Paget, 2019; WHO, 2018). This is an issue as crowded conditions in nursing homes create an environment of high transmissibility (CDC, 2018). Immunocompromised individuals also have a greater risk associated with influenza due to a weakened immune response (WHO, 2018). Each of these vulnerable populations experience higher mortality rates due to influenza than the general population (WHO, 2018).

Mortalities and poor health outcomes due to infection by pathogens are also higher in communities experiencing poverty or social marginalization (WHO, 2021). Although this disparity is recognized, minimal efforts have been allocated to combat the issue (Quinn, 2014). As a result, low-income households experience greater hospitalizations, infections, and deaths from influenza (Quinn, 2014). For example, in 2009, in the UK, those with the lowest incomes had an influenza mortality rate three times higher than middle to high income families (Quinn, 2014). Social factors, such as race, also correlate with poor health outcomes and higher mortalities in a population. Rates of hospitalizations are generally higher for minority groups with admission rates per 100,000 being 68.8 for African Americans, 48.7 for American Indians and Alaska Natives, 44.5 for Hispanics or Latinos, and 38.1 for Whites (CDC, 2021). Similar trends based on ethnicity are revealed with higher mortalities and intensive care unit admissions for minority groups (CDC, 2021). The wealth of a country also impacts the citizens of

that country's health outcomes. A flu pandemic is very costly, as the World Health Organization predicts the United States would spend $60 billion each year of a pandemic and $4.5 billion is spent each year to prepare for a pandemic (WHO, 2021). Poor countries lack the finances to prepare for and combat a pandemic (WHO, 2021). As a result, the least developed countries and low-income social groups are impacted the most by poor health outcomes.

Transmission, Infection, and Immune Reactions

Influenza, a zoonotic virus, poses a high threat to both animal and human populations (Moreira, 2021). Zoonotic viruses jump from animals to humans; during this jump, the virulence of the pathogen usually increases (Moreira, 2021). High virulence contributes to the rapid transmission within the human population. Occasionally, fomites are responsible for transmission, as the flu virus may be present on a surface; if a person touches the contaminated object and touches their eyes, mouth, or nose they may become inoculated with influenza (CDC, 2018). Most often, the disease is directly transmitted by respiratory droplets when an infected individual coughs, sneezes, or talks (WHO, 2018). The droplets land on the mouths and noses of nearby individuals and are inhaled into the lungs (CDC, 2018). This type of transmission follows the epidemic pattern of propagated transmission, utilizing humans as a host for infection (CDC, 2012). These outbreak types have infection levels that peak during the incubation period, which is two days on average, but can range from one to four days after a group has been exposed to influenza (CDC, 2012; WHO, 2018). After incubation, during the prodromal period, one may have mild to no symptoms and contribute to the transmission of the virus before one even knows they are sick (CDC, 2018). Next, the highest viral titers occur within one to two

days of infection and symptoms are apparent; once this point is reached, high levels of virus are maintained for up to six days where most of the susceptible epithelial cells are infected, then the virus begins to decline (Smith, 2018). During this time of infection, bacterial and viral pathogens can cause secondary infections that increase disease severity (Smith, 2018).

Influenza's high disease severity is due to the body's lack of immunity against different strains of the influenza virus (WHO, 2018). These strains arise due to rapid mutation of surface glycoproteins causing antigenic drift (Potter, 2001). Mutations of these antigenic surface molecules allow humoral immune response evasion because the antibodies responding to the original virus are no longer effective against the newer variants (Abbas, 2020). Influenza surface glycoproteins include haemagglutinin and neuraminidase which are normally targeted by antibodies when immunity has been developed, and mutations render the influenza virus unrecognizable even if an individual has been previously infected by a different, related variant (Potter, 2001). The highly transmissible and pandemic causing Influenza A has different subtypes due to haemagglutinin and neuraminidase mutations, including the currently circulating viruses of H1N1 and H3N2 (WHO, 2018).

When the influenza A virus infects a host, it causes an acute infection of the upper and lower respiratory tracts (Smith, 2018). The immune system attempts to prevent this infection. Upon one's first exposure, innate immunity provides the first line of defense (Abbas, 2020). The virus must first invade physical barriers, such as the epithelium which has tight junctions covered by mucous or keratin to mechanically prevent infection (Abbas, 2020). The influenza virus penetrates mucus membranes by removing them, as

neuraminidase glycoproteins cleave sialic acids that make up the mucus, which allows contact with underlying cells (Zanin, 2016). The viral envelope then binds respiratory cells when HA attaches to sialic acid on the host cell membrane (Moreira, 2021). Next, the viral envelope fuses with the host membrane, releasing the nucleocapsid to the cytoplasm where it is uncoated, replicated, translated, and released to infect healthy cells (Moreira, 2021). After physical barriers have been bypassed, the virus enters the tissues (Abbas, 2020). Receptors of the innate immune system recognize thousands of molecular patterns including damage-associated molecular patterns (DAMPS) and pathogen-associated molecular patterns (PAMPS), such as the single-stranded RNA of influenza (Abbas, 2020). After these patterns are recognized, the innate immune system protects the body by causing inflammation and activation of dendritic cells, macrophages, neutrophils, and plasma proteins to eliminate extracellular microbes, phagocytize and destroy pathogens, cause damage and tissue necrosis, and initiate tissue repair (Abbas, 2020). Once the virus has infected a cell, the innate intracellular response attempts to eliminate cells through the Natural Killer (NK) cell response (Abbas, 2020). NK cell granule secretions cause infected cells to undergo apoptosis, macrophages that phagocytize infected cells are activated by Interferon γ (IFN-γ), and type I Interferon (IFN) cytokines induced by Rig-Like Receptor recognition of viral RNA block viral replication (Abbas, 2020). Lastly, the innate immune system defends against viruses by aiding the activation of the adaptive immune system, which targets specific antigens (Abbas, 2020).

Upon subsequent exposures the immediate innate response does not respond differently; however, the adaptive response is quicker and stronger. This is because

during the first exposure the humoral and cell-mediated responses must be activated and pathogen-specific cells must grow in numbers and mature to respond to the virus, resulting in the adaptive response taking seven to ten days to develop (Abbas, 2020). The primary cell-mediated response targets protein antigens via T cells. Recognition occurs by two pathways for exogenous and endogenous antigens. Exogenous antigens are internalized by antigen-presenting cells, such as B-cells and macrophages, that present the antigen by the MHC Class II pathway where CD4+ T-cells are presented with unfolded proteins and co-stimulatory molecules from antigen-presenting cells to become activated (Abbas, 2020). After activation, these cells proliferate and function to activate macrophages to eliminate the exogenous antigen (Abbas, 2020). Endogenous antigens are found within cells and presented through the Class I MHC pathway for all nucleated cells (Abbas, 2020). CD8+ T-cells, which are the primary response against viruses, then recognize these endogenous antigens and cause the cell to undergo apoptosis through degranulation (Abbas, 2020). During the primary humoral response, B cells must be activated before they respond to antigens. B-cells can be T-independent or T-dependent and each is activated differently and respond to different molecules. Most B-cells are T-dependent and respond to proteins, such as HA and NA on influenza (Abbas, 2020). T-dependent activation requires two signals for activation, including CD4+ T-cell stimulus and antigen binding to the variable Fab portion of the B-cell receptor (Abbas, 2020). The interaction of stimulatory molecules on the B-cell and T-cell leads to class switching and affinity maturation. Class switching occurs as regions downstream from the variable domain are spliced to obtain M, D, A, E, and G antibody classes (Abbas, 2020). These antibody classes each play different roles in defending against viruses like influenza by

eliminating antigens. IgM plays a major role in the activation of the classical complement pathway, which leads to inflammation, opsonization, and cell lysis; IgG is important for neutralization, opsonization, and antibody-dependent cellular cytotoxicity; and IgA prevents infection as it is secreted in the mucus (Abbas, 2020). Affinity maturation occurs as variable regions that bind antigen undergo rapid mutation. Regions with a higher affinity to the pathogenic antigen are selected, resulting in a more efficient response (Abbas, 2020). T-independent B-cells are also important response mechanisms as they recognize polysaccharides, lipids, and nucleic acids (Abbas, 2020). Activation occurs as B-cells encounter non-protein antigens that cross-link on the antibody receptor surfaces, resulting in the rapid production of IgM (Abbas, 2020). After the second exposure, the adaptive response retains memory B cells and memory T cells. These memory cells allow for the rapid proliferation of T-cells and B-cells upon repeated exposures to antigens, creating a quick response as early as two to seven days after exposure (Abbas, 2020).

Vaccination and Immunologic Reactions

Natural exposure to influenza antigens causes natural active immunity consisting of B-cell and T-cell responses against the virus. B-cell and T-cell immunity can also be induced through artificial active vaccination, which induces a primary immune response (Clem, 2011). This type of vaccination occurs when live-attenuated viruses are administered by intranasal pathways or dead-inactivated viruses are injected intramuscularly (Clem, 2011). These antigens stimulate the immune system as they are internalized by antigen-presenting cells, allowing for the activation and differentiation of B-cells and T-cells (Clem, 2011). These B-cells and T-cells become memory cells after

the antigen is eliminated, creating a long-lasting response (Clem, 2011). Upon subsequent exposure to natural antigens, the body will respond faster and stronger to eliminate the antigen and decrease the negative effects of influenza (Clem, 2011).

Natural passive immunity also protects against the influenza virus. Natural passive immunity is humoral antibodies acquired as the result of transfer from mother to fetus (Clem, 2011). This concept is particularly important for the vulnerable population of newborns because deaths are fifteen times higher during the first four weeks after birth because the immune system is not developed so they are highly vulnerable to disease (WHO, 2011). The transfer of IgA and IgG through the colostrum and milk after birth provides short-term protection for these newborns until their immune system is mature and they can be vaccinated (Clem, 2011). When newborns with maternal immunity are challenged with the influenza virus, epithelial barriers with IgA in mucus membranes prevent viral entry (Abbas, 2020). If the virus bypasses anatomical barriers, IgG within the tissues and blood bind influenza epitopes to neutralize the virus and prevent cellular entry so the virus cannot spread (Abbas, 2020). IgG also plays an important role in eliminating the antigen as its functions include neutralization, opsonization, and antibody-dependent cellular cytotoxicity (Abbas, 2020). IgG plays a role in opsonization because it tags viral particles for phagocytosis, allowing phagocytes to ingest and destroy the pathogen (Abbas, 2020). IgG also triggers antibody-dependent cellular cytotoxicity, causing natural killer cells to degranulate and destroy cells with endogenous antigens (Abbas, 2020). Both IgA and IgG play a major role in the prevention of infection, so newborns with no antibody protection against influenza are much more susceptible to infection and severe disease outcomes (Abbas, 2020). Since active vaccination is not

recommended until six months of age, early immunity through passive antibody transfer is essential to survival as the number of respiratory illnesses, fever, and hospitalization rates in newborns are decreased for those with vaccinated mothers (Kalenik, 2014).

Mothers without protection against influenza are also more susceptible to infection and severe disease outcomes. During pregnancy, mothers are immunosuppressed to prevent the immune system from attacking foreign fetal cells (Becker, 2012). As a result, T-regulatory cells inhibit reactions that attack foreign and self-antigens (Becker, 2012). This protects the fetus from conditions like spontaneous miscarriage or preeclampsia; however, it increases pregnant women's vulnerability to diseases, such as influenza (Becker, 2012). Flu-related complications, including pneumonia, occur at higher instances for pregnant women than in the general population (Becker, 2012). Since this population is at risk, the CDC states pregnant women should be vaccinated against seasonal influenza (Becker, 2012). When the vaccinated population encounters influenza antigen, even with immunosuppression, T-cells and B-cells will proliferate to eliminate the antigen; whereas the unvaccinated population experiences a slow primary response that is more likely to be halted by regulatory T-cells (Abbas, 2020). The immune response in those who have been vaccinated protects against primary infection and decreases the possibility of secondary infection because they experience a shorter period between infection and convalescence (Abbas, 2020). Maternal health is important because poor health is linked with child mortality (WHO, 2011). Providing care to mothers, including vaccination, during pregnancy increases child survival rates (WHO, 2011). Vaccination prevents fever and illness, which can harm the fetus during pregnancy (CDC, 2018). For example, women who experienced fever during pregnancy

were two times more likely to have a baby with a neural tube defect compared to mothers who did not have a fever (CDC, 2018). This evidence reinforces the importance of vaccination to protect the mother and fetus.

History of Vaccines

Historically, vaccination has been important for controlling the transmission, infection, and severe effects of influenza (Barberis, 2016). The first step to creating these vaccines was the isolation of the influenza virus. This began during the 1918-19 pandemic when scientists believed that an agent different from bacteria was transmitting the disease, and in 1933 the influenza A virus was first isolated from the nasal secretions of human patients (Barberis, 2016). From 1933 to 1936 scientists worked on important laboratory techniques, including in vitro viral transmission, viral growth, viral inactivation, purification by centrifugation, and antibody generation and isolation (Barberis, 2016). The first clinical trials of inactivated vaccines occurred during the mid-thirties (Barberis, 2016). The first of these trials occurred in 1937 with soldiers in England being vaccinated subcutaneously with an inactivated virus isolated from a mouse lung (Barberis, 2016). The second trial of an inactivated influenza vaccine, in 1938, rendered the US military protected from influenza. In the 1940s, the first widespread vaccine was a monovalent influenza A vaccine, which means it contained one subtype of inactivated influenza A (Barberis, 2016). Clinical trials showed these vaccines were effective, but they did not protect against a newly emerging influenza B, so a new vaccine was needed (Barberis, 2016). In 1942, a bivalent vaccine with influenza A and B was utilized (Barberis, 2016). Due to the mismatch seen between influenza A and B, the World Health Organization (WHO) created a global surveillance system with several

countries in 1952 (Barberis, 2016). The systems allowed vaccines to be made based on the previous season's viral influenza (Barberis, 2016). Due to the 1968 pandemic, split trivalent vaccines were created as they produced fewer reactions than whole virus vaccines, especially for children (Barberis, 2016). These vaccines were less immunogenic and yielded less protection than whole virus vaccines, so highly immunogenic, tolerable subunit vaccines containing isolated and purified HA and NA surface antigens were created using genetic reassortment in 1976 (Barberis, 2016). Live attenuated vaccines were also important for early vaccination with clinical trials occurring in 1935-1941, and in 1949 cell cultures were used for attenuated viral growth (Barberis, 2016). This type of vaccine was authorized in the United States in 2003 for intranasal use (Barberis, 2016). Around 2009 adjuvants, such as alum and oil in water, were applied to increase the antigenicity of vaccines to increase immune reactions (Barberis, 2016). Another new technique, developed in 2011, is the intradermal delivery of vaccines to involve dermal antigen-presenting cells for increased immunological response (Barberis, 2016). Next, the quadrivalent vaccine, an inactivated, split vaccine composed of two influenza B and two influenza A strains, was approved by the United States in 2012 to decrease mismatch and maintain immunogenicity (Barberis, 2016). To increase immunogenicity, in 2013, the FDA approved a recombinant trivalent vaccine with a threefold higher HA dose. Finally, scientists are currently developing universal vaccines that target conserved M2e or hemagglutinin stalk proteins and exploit T-cells to cause broader antibody responses (Barberis, 2016).

Current vaccine types are live-attenuated, recombinant, and dead-inactivated vaccines (WHO, 2018). The WHO currently recommends the quadrivalent vaccine due to

its wider protection against the most representative influenza strains, including H1N1, H3N2, BYamagata, and BVictoria (WHO, 2018; WHO 2020). Injectable forms of the influenza vaccine include recombinant and dead-inactivated vaccines, and intranasal forms include live-attenuated vaccines (WHO, 2018). Recombinant vaccines are produced using recombinant DNA technology, so it does not require virus grown in eggs (CDC, 2021). Inactivated vaccines are created using heat, cold, irradiation, or chemicals to destroy a pathogen and create a stable vaccine that can be easily transported (Clem, 2011). Live-attenuated vaccines contain a weakened virus, such as cold-adapted live attenuated influenza vaccines (Clem, 2011). Since these vaccines contain the live pathogen, they elicit a stronger and longer-lasting immune response (Clem, 2011). Other important vaccines to recognize are flu vaccination by jet injector approved for ages 18-64, high-dose flu vaccine with four times the antigen for those 65 and older, and cell-based vaccines cultured from a mammalian origin (CDC, 2021).

Surveillance is essential for these vaccines to be effective. The quadrivalent vaccine has two influenza A and two influenza B subtypes, but these must be circulating in the population for the vaccines to be protective (WHO, 2018). Surveillance and predictions are utilized as epidemics seen in the Eastern and Southern hemispheres form the basis of the vaccine for Europe and North America (Potter, 2001). Sometimes these predictions are inaccurate due to different subtypes affecting North America and Europe, so the vaccine is less effective (Potter, 2001). As a result, the flu season experiences increased transmission, mortalities, and morbidities (Potter, 2001). The number of mortalities and morbidities also depends on the number of people who are vaccinated. In the United States, the percentage of adults over eighteen receiving the vaccine was

48.1%, while the number of children aged six months to seventeen years was 52.5% over the past year (CDC, 2022). Due to higher vaccination rates, the United States has 1.8 deaths per 100,000 due to influenza compared to 2.29 per 100,000 in South Africa (CDC, 2022; Gul, 2018). This difference is because antigens within the vaccine stimulate a primary response against the influenza virus, allowing a faster and stronger secondary response when exposed naturally (Abbas, 2020). Stronger immune responses due to vaccination reduce the risk of flu illness between 40-60%; those infected experience a 31% lower risk of death and 26% lower risk of severe illness (CDC, 2021). Overall, vaccination prevents death and hospitalization, while decreasing the time with illness in those infected with influenza.

The Pig Model

The pig model is used due to its similarities with humans as newborns lack antibodies when they are born due to having a non-invasive placental barrier, so antibodies must be transferred through a mother pig's milk (Salmon, 2009). These antibodies provide lactogenic immunity to local pathogens until vaccination is recommended (Salmon, 2009). Antibodies are transferred through breastfeeding with IgG and IgM transfer through the colostrum and IgA transfer through the milk (Salmon, 2009). Colostrum is a nutrient-rich fluid produced for several days after birth, while milk is produced throughout breastfeeding (Uruakpa, 2002). IgG, IgM, and IgA are transferred to the newborn through the gut as the antibodies undergo transcytosis into enterocytes to provide immunity in the piglet (Salmon, 2009). Along with similarities to humans, the species are relatively easy to care for, have large numbers of offspring, and are low-cost (Salmon, 2009). Large litter sizes are important to decrease the effect of confounding

variables and deviations on research outcomes (Langmeier, 2019). Low-cost specimens and ease of care are important to ensure inexpensive, feasible research.

Clinical Relevance

The WHO and CDC recommend that pregnant women get vaccinated because it is safe and effective (Becker, 2012; WHO, 2018). Clinical advocacy for the vaccine has been increasing as clear scientific research supports the safety and efficacy of the flu vaccine in pregnant women (CDC, 2021). The CDC analyzed reports of the Vaccine Adverse Reporting System, finding no link between vaccination during pregnancy and complications or adverse fetal outcomes (CDC, 2021). Large studies using CDC vaccine safety data found no link between spontaneous abortion, miscarriage, adverse obstetric events, premature delivery, and birth defects and vaccination (CDC, 2021). Evidence of vaccine benefits include decreasing pregnant women's chances of being hospitalized due to influenza by 40%, decreasing the risk of fetal birth defects due to fever, and protecting newborns from influenza post-birth (Lamppa, 2021). Since the vaccine is both safe and effective, it should be implemented into prenatal care before each pregnancy as it is the best way for pregnant mothers to protect themself and their babies from flu-related complications (CDC, 2020). Without this vaccination, newborns are highly vulnerable to influenza until they can receive the vaccination at the age of six months (Wild, 1999).

Purpose

Since no vaccination exists for infants under six months old, other methods such as passive immunity must be explored (Wild, 1999). Studies have shown that maternal influenza vaccination is associated with fewer respiratory illnesses during the first six months of the life of a newborn (Vanderlubbe, 2017). We know that both IgA and IgG

antibodies are important for protection against influenza virus infection (VanDerLubbe, 2017). However, we do not know whether decreased respiratory illness in infants is related to the transfer of IgA in breastmilk or IgG from colostrum (Albrecht, 2020). To help determine how infants can be protected, we tested the passive transfer of a universal influenza vaccine using a pig model and researched the mechanism of transfer. This model allowed us to determine whether the transfer of IgA from the mother's milk and IgG from the colostrum provided the antibody levels required for protection. Utilizing research procedures, we tested the hypothesis that if vaccinated mother pigs transfer high levels of antibodies to the newborn, then antibodies will protect influenza-challenged offspring from the influenza virus.

CHAPTER TWO

Materials and Methods

Procedure in Pigs

Testing whether antibodies are transferred was done by testing antibody levels and the presence of the virus in nasal swabs using hemagglutination assays for influenza challenged piglets. Using this procedure, we test whether transferred antibodies are protecting pigs from infection. This protection requires a high level of antibodies, so we determine whether these antibodies are passed down and if they are produced in a large enough quantity to provide protection. Research began as four mother pigs (X6912, X6913, X6914, and X6915) were vaccinated twice with the HA-129 broad immunity influenza vaccine, and four mother pigs (X6721, X6910, X6704, and X6911) were vaccinated once with PBS and once with the HA-129 vaccine prior to challenge with viruses expressing the IA and IL influenza virus hemagglutinins. The pigs received their first vaccine between 4/30 and 5/2 and their second vaccine between 5/15 and 5/17. The mothers were farrowed between 6/23-6/25 and piglets were challenged with influenza virus three days post-birth with the X6721, X6704, X6912, and X6914 groups challenged with IA influenza virus hemagglutinins and X6910, X6911, X6913, and X6915 groups challenged with IL influenza virus hemagglutinins. Nasal swabs were collected on day 0, day 3, and day 5 post-inoculation.

MDCK Growth Media

MDCK growth media is essential to support the growth of Madin-Darby Canine Kidney (MDCK) cell lines. The procedure to make MDCK growth media occurred under a sterile hood using an aseptic technique, beginning by attaching a 500 mL sterile filter

with a pump system to a one-liter bottle. Next, 100 mL of 10X Minimum Essential Medium (MEM), 50 mL of Heat-inactivated Fetal Bovine Serum (FBS), 10 mL of Antibiotic/ Antimycotic Solution, 10 mL of MEM Vitamin Solution, 10 mL of L-Glutamine, 1 mL of Gentamicin, and about 320 mL of distilled water was added to the filter; the pump was turned on for filtration. After this solution was filtered and the pump was stopped, 30 mL of $NaHCO_3$ and 470 mL of distilled water were added and filtered. The complete solution appeared red and was checked for correct pH based on color. MDCK growth media was stored in a 4℃ refrigerator for later use.

MDCK Infection Media

MDCK infection media is important to promote viral infection of MDCK cell lines. The procedure to make MDCK infection media occurred under a sterile hood using an aseptic technique, beginning by attaching a 500 mL sterile filter with a pump system to a one-liter bottle. Next, 100mL of 10X MEM, 40mL of a 7.5% Bovine Serum Albumin (BSA) in water, 10mL Antibiotic/Antimycotic solution, 10 mL MEM Vitamin Solution, 10 mL L-Glutamine, and 330 mL of distilled water was added to the filter; the pump was turned on for filtration. After this solution was filtered and the pump was stopped, 30mL $NaHCO_3$ and 470 mL of distilled water were added and filtered. The complete solution appeared red and was checked for correct pH based on color. MDCK infection media was stored in a 4 ℃ fridge for later use.

Cell Culture and Plating Procedure

MDCK cells are used during experimentation due to their high susceptibility to influenza infection (Seitz, 2010). Cell culture and plating procedures began with the growth and maintenance of an MDCK cell line from stock media. These cells are seeded

into a T-75 flask and incubated at 37℃ with 5% CO_2. These cells were then passaged when 90% confluency was reached and maintained in a larger T-150 flask. The passaging procedure from one T-150 to another T-150 began as excess media was removed with a pipet. Next, the cells were washed with 10 mL of PBS and excess PBS was removed. 1 mL of 0.5% trypsin and 9 mL of PBS were added to the flask, and it was incubated at 37℃ with 5% CO_2 for one hour. Then, the cells were pipetted and placed into a corning tube that was centrifuged at 2500 g for three minutes. The remaining PBS and trypsin supernatant was removed, and the pellet was resuspended in 10 mL of MDCK Growth Media. One milliliter of the media was allocated to another T-150 flask with 39 mL of MDCK growth media and placed in the incubator at 37℃ with 5% CO_2 to continue MDCK cell growth for future experiments. 100 µL of resuspended cells from the remaining 9mL solution was added to a small test tube with 400 µL of growth media and 500 µL of trypan blue. Ten µL of this solution was loaded into the hemocytometer and cells were counted under the microscope. Growth media was added to dilute the remaining solution to reach a concentration of 3×10^5. The solution was added to 48 well plates with a concentration of 3×10^5 cells in 1 mL of solution for each well. The plates were incubated at 37℃ with 5% CO_2 overnight to achieve 85-90% confluency.

Infection Procedure (TCID 50)

To determine whether the influenza virus was present, a TCID50 test was completed to reveal the concentration where 50% of the plated cells were infected. The first step of the procedure was performing 10-fold serial dilutions to dilute viral stock (nasal wash) using MDCK infection media. 900 µL of infection media was added to ten test tubes as they are placed in an ice bath. Next, 1 mL of viral stock was added to tube 1

and vortexed; it was serially diluted by taking 1 mL from the previously vortexed tube and transferring it to the following tube with 900 μL of media followed by vortexing.

The incubated 48-well plates were removed and placed in the sterile hood. Media was aspirated from each of the wells with a glass pipette. Each well was washed twice with 250 μL of sterile 1X PBS and aspirated. 50 μL of the diluted virus was added to each well with media added first, then adding the lowest (10^{-12}) to the highest dilutions (10^{-2}). A1-A4 contained media, B1-B4 contained 10^{-2} concentration, C1-C4 contained 10^{-3} concentration, D1-D4 contained 10^{-4} concentration, E1-E4 contained 10^{-5} concentration, F1-F4 contained 10^{-6} concentration, A4-A8 contained 10^{-7}, B4-B8 contained 10^{-8} concentration, C4-C8 contained 10^{-9} concentration, D4-D8 contained 10^{-10} concentration, E4-E8 contained 10^{-11} concentration, and F4-F8 contained 10^{-12} concentration. The plate was incubated at 37℃ with 5% CO_2 tapping every 15 minutes to ensure proper distribution of the viral sample across the monolayer. The virus inoculum was aspirated and 500 μL of MDCK infection media with 1 μg/mL TPCK Trypsin was added to the wells. Plates were incubated at 37℃ with 5% CO_2 for 72 to 96 hours. Cells were observed for cytopathic effects due to the presence of the virus. Each treatment group followed the same procedure.

Preparation of Chicken Red Blood Cells Procedure

Chicken red blood cells (CRBC) with Alsevers solution were resuspended before transfer to conical tubes. 12 mL of CRBC was transferred to 15 mL tubes and centrifuged at 1,338 g for four minutes at 25℃. Supernatant and the white blood cell layer were removed to preserve the CRBC layer. 10 mL of 1X PBS was added to each tube with CRBC and the solution was centrifuged at 800 g for 20 minutes at 25℃. 0.5 mL of

CRBC from these tubes was added to 100 mL of 1X PBS and resuspended. The cells were stored at 4℃ for later HA assay use.

Hemagglutination (HA) Assay

HA assays are used to determine the presence and concentration of the influenza virus in a sample using titers. It utilizes properties of red blood cell binding as influenza viruses bind to RBCs forming a lattice structure (CDC, 2021). This characteristic of hemagglutination keeps RBCs suspended in solution when influenza virus is present as opposed to sinking and forming a pellet when the virus is absent (CDC, 2021). Once 72-96 hours of inoculation was complete, media for two samples were transferred to a ninety-six well plate by pipetting up and down in each well before transferring to the appropriate location. 50 μL of 0.5% CRBC solution was added to each well and the solution remained undisturbed for thirty minutes before reading the results. The results were analyzed for the absence (pellet) or presence (hemagglutination) of influenza virus with a presence at the lowest concentration indicating titer values.

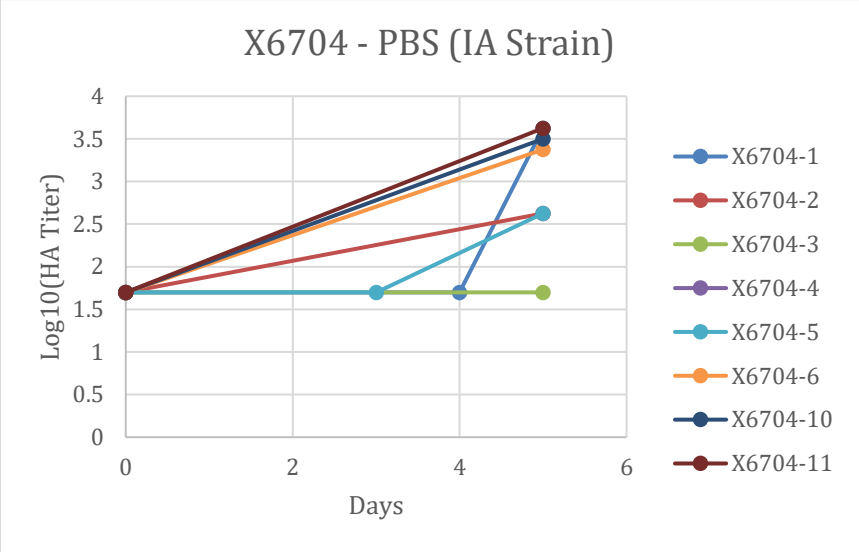
CHAPTER THREE

Results

In challenged piglets, viral titers were obtained via HA assays to confirm and quantify influenza virus presence along with TCID50s. These techniques allowed us to test for the presence of virus in piglets of vaccinated and unvaccinated mothers. Mothers vaccinated with PBS (Figures 1.1-1.8) produced piglets with higher viral titers than influenza vaccinated mothers (Figures 2.1-2.8). Mean titers for Figures 1.1 and 1.2 (X6704) were 2468.5 for non-baseline values, 1662.3 for days 2-5, 903.59 for days 0-5, 50 for day 0, 50 for day 3, 50 for day 4, and 2123 for day 5. Mean titers for Figures 1.3 and 1.4 (X6721) were 50 for days 0-5, 50 for day 0, 50 for day 3, 50 for day 4, and 50 for day 5. Mean titers for Figures 1.5 and 1.6 (X6910) were 133.35 for non-baseline values, 92.9 for days 2-5, 65.63 for days 0-5, 50 for day 0, 66.67 for day 2, and 133.35 for day 5. Mean titers for Figures 1.7 and 1.8 (X6911) were 50 for days 0-5, 50 for day 0, and 50 for day 5. The overall mean titer value for PBS vaccinated mothers on days 2-5 was 691.8. Mean titers for Figures 2.1 and 2.2 (X6912) were 1000 for non-baseline values, 525 for days 2-5, days 0-5, 50 for day 0, and 525 for day 5. Mean titers for Figures 2.3 and 2.4 (X6913) were 567.5 for non-baseline values, 285.2 for days 2-5, 173.22 for days 0-5, 50 for day 0, 50 for day 3, and 308.769 for day 5. Mean titers for Figures 2.5 and 2.6 (X6914) were 50 for days 0-5, 50 for day 0, and 50 for day 5. Mean titers for Figures 2.7 and 2.8 (X6915) were 50 for days 0-5, 50 for day 0, 50 for day 3, and 50 for day 5. The overall mean for HA-129 vaccinated mothers on days 2-5 was 258.1. These means reveal lower titers and greater protection for mothers receiving HA vaccination.

Figure 1.1: Graph of Titer Values for Piglets of PBS Vaccinated Mother X6704 Days 0-5

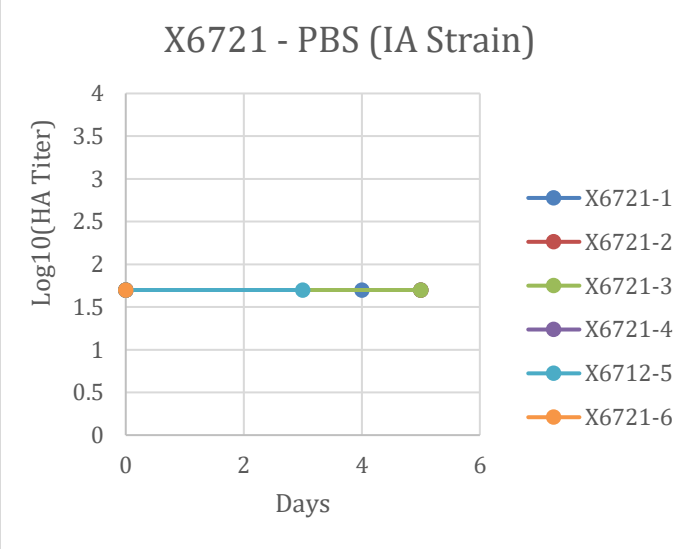
Figure 1.2: Table of Titer Values for Piglets of PBS Vaccinated Mother X6704 Days 0-5



X6704 - PBS (IA Strain)		
Sample	Day	Titer
X6704-1	0	50
X6704-1	4	50
X6704-1	5	4216.97
X6704-2	0	50
X6704-2	5	421.7
X6704-3	0	50
	5	50
X6704-4	0	50
X6704-5	0	50
	3	50
	5	421.7
X6704-6	0	50
	5	2371.37
X6704-10	5	3162.27
	0	50
X6704-11	5	4216.97
	0	50

Figure 1.3: Graph of Titer Values for Piglets of PBS Vaccinated Mother X6721 Days 0-5

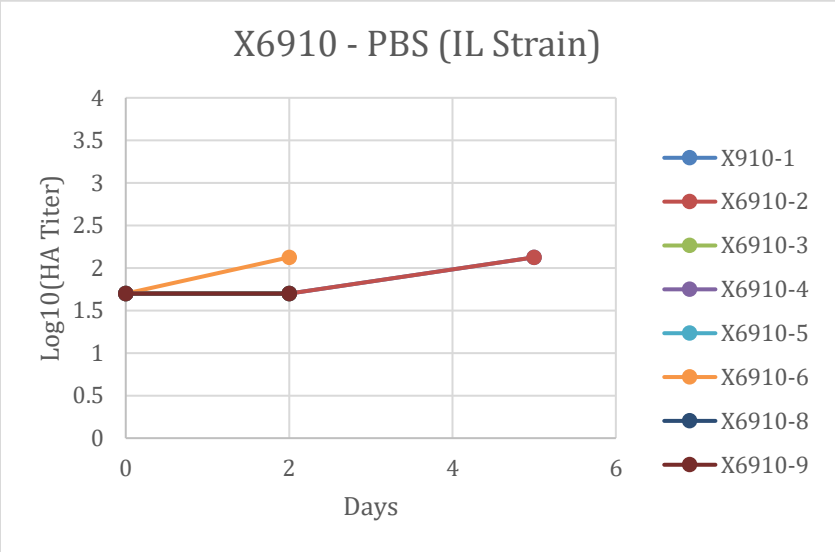
Figure 1.4: Table of Titer Values for Piglets of PBS Vaccinated Mother X6721 Days 0-5



X6721 - PBS (IA Strain)		
Sample	Day	Titer
X6721-1	0	50
	4	50
	5	50
X6721-2	0	50
	5	50
X6721-3	0	50
	5	50
X6721-4	0	50
X6721-5	0	50
	3	50
X6721-6	0	50

Figure 1.5: Graph of Titer Values for Piglets of PBS Vaccinated Mother X6910 Days 0-5

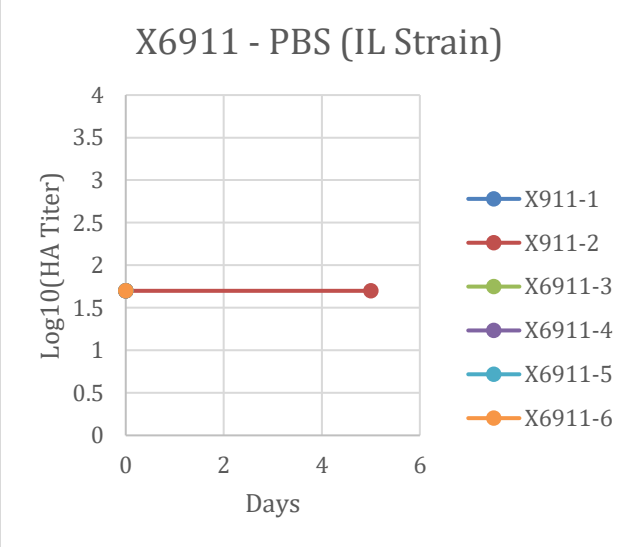
Figure 1.6: Table of Titer Values for Piglets of PBS Vaccinated Mother X6910 Days 0-5



X6910 - PBS (IL Strain)		
Sample	Day	Titer
X6910-1	0	50
	2	50
	5	133.35
X6910-2	0	50
	2	50
	5	133.35
X6910-3	0	50
	2	50
X6910-4	0	50
X6910-5	0	50
X6910-6	0	50
	2	133.35
X6910-8	0	50
	2	50
X6910-9	0	50
	2	50

Figure 1.7: Graph of Titer Values for Piglets of PBS Vaccinated Mother X6911 Days 0-5

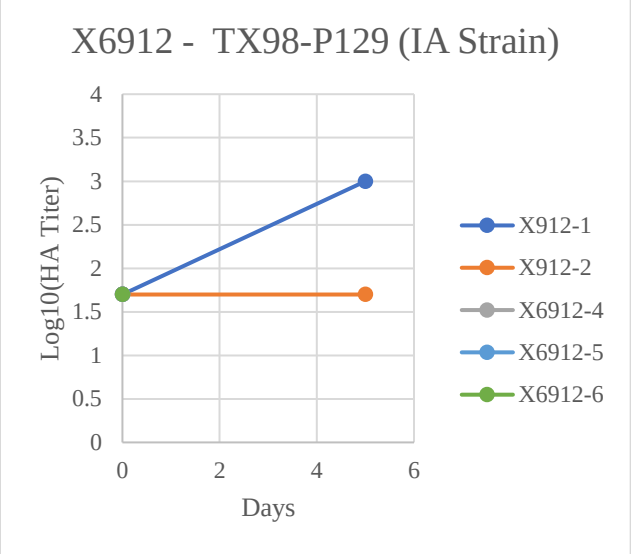
Figure 1.8: Table of Titer Values for Piglets of PBS Vaccinated Mother X6911 Days 0-5



X6911 - PBS (IL Strain)		
Sample	Day	Titer
X6911-1	0	50
X6911-2	0	50
	5	50
X6911-3	0	50
X6911-4	0	50
X6911-5	0	50
X6911-6	0	50

Figure 2.1: Graph of Titer Values for Piglets of HA-129 Vaccinated Mother X6912 Days 0-5

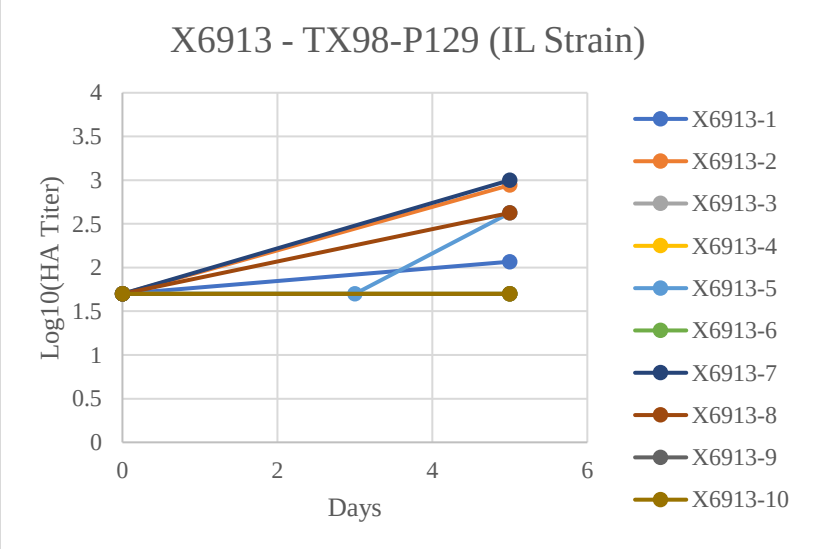
Figure 2.2: Table of Titer Values for Piglets of HA-129 Vaccinated Mother X6912 Days 0-5



X6912 - TX98-P129 (IA Strain)		
Sample	**Day**	**Titer**
X6912-1	0	50
	5	1000
X6912-2	0	50
	5	50
X6912-4	0	50
X6912-5	0	50
X6912-6	0	50

Figure 2.3: Graph of Titer Values for Piglets of HA-129 Vaccinated Mother X6913 Days 0-5

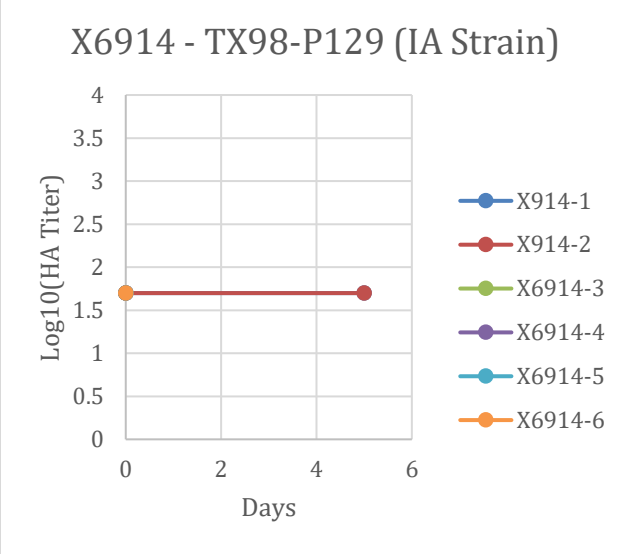
Figure 2.4: Table of Titer Values for Piglets of HA-129 Vaccinated Mother X6913 Days 0-5



X6913 – TX98-P129 (IL Strain)		
Sample	**Day**	**Titer**
X6913-1	0	50
	5	116.68
X6913-2	0	50
	5	877.61
X6913-3	0	50
	5	50
X6913-4	0	50
	5	50
X6913-5	0	50
	3	50
	5	421.7
X6913-6	0	50
	5	50
X6913-7	0	50
	5	1000
X6913-8	0	50
	5	421.7
X6913-9	5	50
	0	50
X6913-10	5	50
	0	50

Figure 2.5: Graph of Titer Values for Piglets of HA-129 Vaccinated Mother X6914 Days 0-5

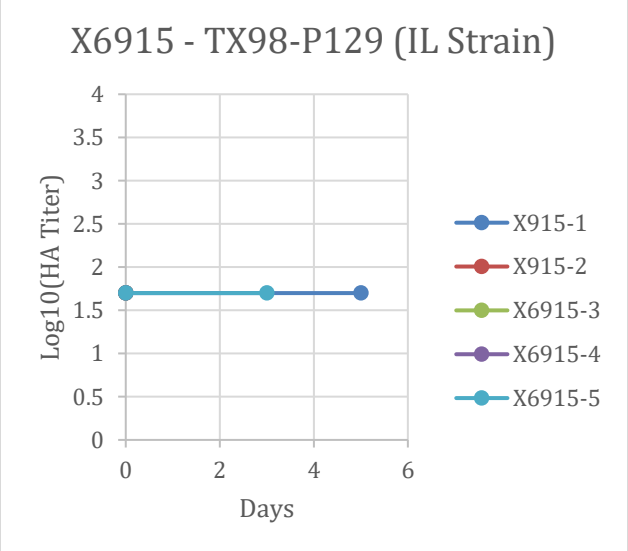
Figure 2.6: Table of Titer Values for Piglets of HA-129 Vaccinated Mother X6914 Days 0-5



X6914 - TX98-129 (IA Strain)		
Sample	**Day**	**Titer**
X6914-1	0	50
	5	50
X6914-2	0	50
	5	50
X6914-3	0	50
X6914-4	0	50
X6914-5	0	50
X6914-6	0	50

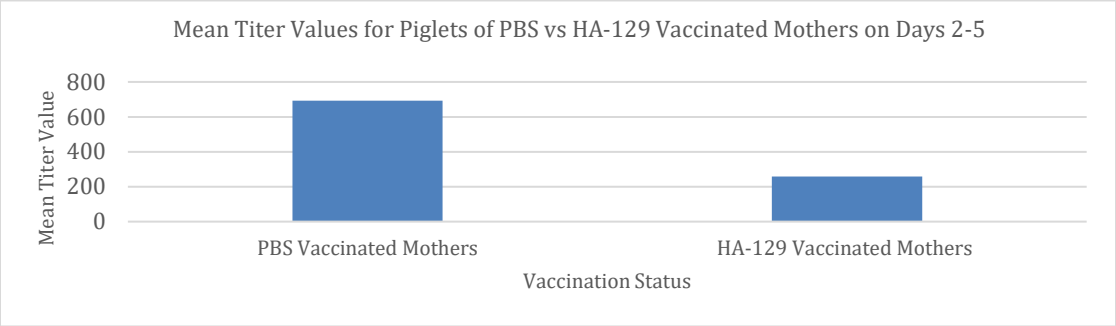
Figure 2.7: Graph of Titer Values for Piglets of HA-129 Vaccinated Mother X6915 Days 0-5

Figure 2.8: Table of Titer Values for Piglets of HA-129 Vaccinated Mother X6915 Days 0-5



X6915 - TX98-P129 (IL Strain)		
Sample	**Day**	**Titer**
X6915-1	0	50
	5	50
X6915-2	0	50
X6915-3	0	50
X6915-4	0	50
X6915-5	0	50
	3	50

Figure 3: Graph of Mean Titers for Piglets of HA-129 vs PBS Vaccinated Mothers on Days 2-5



Along with higher mean values, the data presented showed higher titer values in those vaccinated with PBS vs HA-129. X6704 attained the highest two titer values at 4216.97 for those vaccinated with PBS, while the highest for those vaccinated with HA-129 was 1000. It is interesting to note that piglets with the most positive results in the PBS group (X6704) had high titer values indicating infection, except X6704-3 and X6704-5 which had baseline values. Meanwhile, the piglets with the most positive results in the HA-129 vaccinated group (X6913) had much lower titer values and increased numbers of baseline values (X6913-3, X6913-4, X6913-6, X6913-9, X6913-10), indicating levels of protection. The group with the second-highest infections for PBS vs HA-129 also exhibited greater protection for the HA-129 vaccinated pigs. X6910-PBS shows three infections within piglets with values reaching as high as 133.35 on only day two, while X6912-HA129 exhibits only one breakthrough infection on day 5. Lastly, two groups for PBS and HA-129 vaccination show no levels of infection due to the innate (PBS vaccinated) or adaptive (HA-129) immune system preventing entry and infection.

An unpaired t-test was used to analyze the data and evaluate the two-tailed p-values. The test gave a p-value of 0.2083, and since this value is greater than 0.05, we are unable to accept our hypothesis that if vaccinated mother pigs transfer high levels of antibodies to the newborn, then antibodies will protect influenza-challenged offspring

from the virus. Although the number is not found to be statistically significant, we can note that the p-value is not 0.5, so the data does point to the possibility that the vaccinated group was better protected against influenza. This possibility is supported as the mean of those vaccinated with PBS minus the mean of those vaccinated with HA-129 indicated a value of 433.9019 on days 2-5, indicating much higher titer values for the PBS group overall. The 95% confidence interval of this difference ranges from -252.35 to 1120.15, revealing a much higher possibility the value is greater than one, indicating higher titer values and less protection for the PBS group. Numbers used in these calculations were t = 1.28, df = 38, and standard error of difference = 338.992.

Sources of error prevent us from definitively confirming our hypothesis. First, the data lacks large numbers of sampling on days 2-5 after inoculation due to sample shipping issues during the COVID-19 pandemic, which resulted in unprocessed samples. When these samples were processed in the Ying lab, the trend for higher viral titer values in the PBS group continued with statistical significance. Next, data may be skewed due to the high number of day 0 samples at a baseline of 50 that we used for training purposes. Lastly, error within the vaccination procedure of pigs interferes with outcomes as the study intended to have a true PBS group, but the first vaccination occurred with PBS and the second with HA-129. The study has been repeated with a true PBS group and these samples are currently being processed. Preliminary research in the Ying lab has found significantly increased lung lesions and higher viral titers in the PBS group compared to the HA-129 vaccinated group. Through this research, we can predict influenza vaccination of mothers leads to the protection of piglets through the passive transmission of IgA and IgG antibodies in the milk and colostrum.

CHAPTER FOUR

Discussion

Data Summary and Comparison to Other Research

Our data revealed higher means for viral titer values in the PBS vaccinated group compared to the HA-129 vaccinated group, indicating higher levels of infection in the PBS group. Combined with a P-value of 0.1813 we support the possibility that the vaccinated group was better protected. Several studies support this possibility in mice, chickens, and humans. Findings within a study of mice supported the idea that pregnant women are immunocompromised as immune responses in pregnant female mice were lower than in non-pregnant female mice (Vanderlubbe, 2017). The study revealed maternal antibody transfer with longevity increasing as the mother received more vaccines, supporting our research for maternal antibody transfer (Vanderlubbe, 2017). When pups were weaned at three weeks of age, antibody levels started to decline, supporting the idea that important IgA and IgG antibodies that protect against influenza are transferred through the milk and colostrum (Vanderlubbe, 2017). This concept is confirmed as research finds the status of vaccination of the mouse the offspring suckles on is much more important than the vaccination status of the mother (Kalenik, 2014). Further, the research conducted by Vanderlubbe et al. (2017) found maternal passive immunity against the influenza virus does not interfere with future active vaccinations. Overall, research with mice supports our prediction that influenza vaccination of mothers leads to the protection of piglets through the passive transmission of IgA and IgG antibodies in the milk and colostrum. Chickens do not feed their young via milk or colostrum. A study of maternal immunity in chickens found with H5N1 inoculation,

those with vaccinated mothers lived only a few days longer than control chicks with only three out of thirty-two surviving, so vaccination of mothers did not significantly affect survival time (Maas, 2011). Further, of the chickens with maternal immunity at birth, antibody titers and protection quickly diminished, revealing lower results for protection than in our pig and the mouse study (Maas, 2011). This study reveals the importance of IgA and IgG transfer through milk and colostrum for the continued, effective protection against the influenza virus. Clinical studies on humans also advocate for the dual protective effects of influenza vaccination for the mother and fetus/newborn. A pooled analysis of inactivated influenza vaccine in pregnant human mothers found 50% efficacy in mothers and 35% efficacy in newborns for protection against laboratory-confirmed influenza (Azziz, 2021). Each of the studies in mice, chickens, and humans support the transfer of important IgA and IgG antibodies in colostrum and milk, reinforcing our prediction of IgA and IgG transfer from mothers to protect piglets challenged with the influenza virus.

Universal Vaccination and Humans

While the maternal transfer of antibodies may protect infants from severe infection during the next pandemic, universal influenza vaccination may prevent the recurrence of future pandemics and epidemics. Currently, there is a threat of G1 and G4 influenza virus emergence with the potential to cause a pandemic or an epidemic. Swine surveillance indicates these subtypes contain triple reassortment derived internal genes, the ability to bind human-type receptors, the ability to produce increased progeny in human epithelial cells, and high aerosol transmission rates which aid the ability of the virus to jump to humans; however, the G1 virus exhibited decreased ability to produce

progeny in epithelial cells compared to the G4 virus (Sun, 2020). In infected ferrets, the G1 virus caused mild clinical signs, whereas the G4 virus caused severe clinical symptoms (Sun, 2020). The potential of high transmission rates and severe infection abilities creates the concern of a future G4 H1N1 pandemic (Sun, 2020). Another cause of concern is the current influenza vaccine does not provide antigen cross-reactivity with the G1 and G4 influenza viruses (Sun, 2020). In a study of 20 serum samples from 4-year-old children vaccinated with a trivalent vaccine, no serum samples reacted with the G1 or G4 H1N1 viral strains (Sun, 2020). In addition, serological surveillance of swine workers indicated 10.4% were infected with the G4 H1N1 virus and 6.5% were infected with the G1 H1N1 virus, indicating the presence of the virus in the human population (Sun, 2020). This increased infectivity is concerning as the population is not immune to these viruses, increasing their ability to replicate within the host and adapt to increase virulence (Sun, 2020). Utilizing the traditional vaccine would require reformulation with G1 and G4 antigens to protect against infection (NIH, 2021). Vaccination with a universal vaccine, such as the HA-129 influenza vaccine, can prevent a pandemic (such as the G1 and G4 H1N1 threat) by providing broad protection against these strains. Our broad-immunity HA-129 universal influenza virus was tested in pigs, but the National Institutes of Health is launching its first human trial of a universal nanoparticle influenza vaccine to provide long-lasting protection against multiple flu virus strains (NIH, 2021). Within animals, this vaccine prompted a robust antibody response (greater than the commercial vaccine) to HA components in monkeys, mice, and ferrets (NIH, 2021). Furthermore, it outperformed the traditional vaccine in producing protective antibodies against subtypes not found in the vaccine (NIH, 2021). Other universal vaccines target

conserved viral stocks instead of the globular HA head that undergoes antigenic variation (Corona, 2020). Stalk targeting creates the potential to protect against novel pandemic influenza strains, preventing the emergence of viruses, including G1 and G4 H1N1 in the human population. In addition to preventing the emergence of the virus, the universal vaccine would decrease influenza escape which often leads to greater pathogenicity (Estrada, 2019). The decrease in vaccine escape is due to targeting genetically conserved components of influenza to create a long-lasting, robust, and widespread antibody and T-cell response (Estrada, 2019).

The universal vaccine increases pandemic preparation by having a viable vaccine in clinical trials that could respond to wide ranges of influenza virus subtypes capable of causing a pandemic. In contrast, vaccines capable of responding to COVID antigens in humans had little development before the pandemic. The WHO ensured preparation to distribute a vaccine, but the lack of vaccine development eliminated the possibility of early clinical trials and early distribution (WHO, 2020). As a result, the WHO had to respond by ensuring case management and prevention in hospitals (WHO, 2020). A lack of viable vaccines created an urgency for development (Kiszewski, 2021). Funding for innovative vaccine development is essential to respond to these emerging public health threats; however, NIH funding for pandemic threats is inconsistent (Kiszewski, 2021). Most of the $17.2 billion funding for vaccine technologies was allocated to current threats, neglecting the threat of emerging diseases, such as COVID (Kiszewski, 2021). From 2000-2019, select vaccine technologies that contributed to COVID vaccine candidates included $9.65 billion for synthetic vaccines, $5.64 billion for adjuvants, $4.58 billion for DNA vaccines, $4.05 billion for live-attenuated vaccines, $1.56 billion

for viral vector-based vaccines, $1.47 billion for inactivated-virus vaccines, $1.06 billion for TLR9 agonists, $943 million for mRNA vaccines, $583 million for virus-like particles, and $519 million for nanoparticle-based vaccines (Kiszewski, 2021). This funding focused on current threats as $9.18 billion was funded for HIV, $639 million for Ebola, $767 million for coronavirus, $555 million for Zika, and $331 million for Dengue (Kiszewski, 2021). These results reveal little funding was spent on coronavirus and mRNA research; however, this research beginning before 2017 was essential to quickly develop a COVID-19 vaccine candidate (Hogan, 2021). As a result of a lack of funding and research preparedness, the world experienced a lack of vaccine development readiness when the pandemic emerged (Kiszewski, 2021). This led to the implementation of social distancing as the best way to avoid viral spread (Kiszewski, 2021).

Social distancing and high rates of infection negatively impacted the economy. Income was reduced, transportation services ceased, and service and manufacturing industries were halted (Front., 2020). The global economic downfall was caused by "loss of life, business closures, trade disruption, and decimation of the tourism industry (Front., 2020)." For example, in China, the production index decreased by 54% during the first February of the pandemic (Front., 2020). By April 2020, the United States experienced a record high unemployment rate of 11% (Front., 2020). In addition, global oil markets and US stock markets declined (Front., 2020). Healthcare finances were also negatively impacted as America's hospitals and healthcare systems lost $50.7 billion per month (Kaye, 2021). The world bank shrunk by 8% and the pandemic cost the global economy two trillion dollars in one year (Kaye, 2021). These examples exhibit the negative economic impacts of the pandemic caused by little economic preparation.

The world is much more prepared to overcome an influenza pandemic. The universal vaccine presents excellent vaccine development readiness as a vaccine candidate for broad influenza pandemics has already been created and placed in clinical trials (NIH, 2021). These vaccines can be implemented for quick vaccine production and broad population immunity. As a result, the wide response to influenza could prevent or drastically decrease the impacts of a pandemic while protecting against seasonal influenza drift variants (Paules, 2018). Having a readily available vaccine should decrease severe illness and infections by decreasing stress on healthcare infrastructure. Further, the constant financial cost of seasonal surveillance and vaccine production would be decreased due to the broad, long-lasting immunity against subtypes of influenza. Lastly, there would be little economic effect as lower morbidities due to protection would decrease the loss of work hours due to infection.

A universal influenza vaccine also increases herd immunity to seasonal variants (Paules, 2018). The long-lasting response eliminates the need to be vaccinated yearly for protection, which may cause some patients to avoid vaccination (Lazar, 2018). For example, low-income groups lack access to healthcare due to an inability to pay for care or take time off work for an appointment, so having a one-time vaccine increases their ability to get vaccinated and protected for long amounts of time (Lazar, 2018). As a result, a universal vaccination would lead to an increased global vaccination rate, increasing herd immunity. Herd immunity increases the protection of the general population and vulnerable groups due to the decrease in host-to-host transmission (WHO, 2020). In addition, the vaccination elicits a strong B-cell and T-cell response, much like the live-attenuated vaccine, but it does not pose a threat to immunocompromised

individuals as the virus cannot be shed with this type of vaccine (Estrada, 2019). Immune protection provided by the universal vaccine addresses the issues caused by influenza, including decreased morbidity and mortality from severe illness (Sah, 2019). Further, those vaccinated and protected from influenza will exhibit decreased symptom severity when infected (Sah, 2019). A decrease in infection and symptom severity is predicted to have a huge positive impact on decreasing healthcare infrastructure influenza burden (Sah, 2019). Replacement of seasonal vaccines is projected to "reduce the influenza burden by 11.5 million cases, 168,703 hospitalizations, 13,161 deaths, and $2.37 billion in direct medical costs (Sah, 2019)." Along with decreasing the burden of direct medical costs, the universal vaccine would decrease the general economic burden of influenza epidemics and pandemics. Productivity losses due to infection would drastically decrease because of the predicted high herd immunity (Sah, 2019). Overall, the universal vaccine addresses the issues of pandemic prevention and preparedness, increasing immunity against the influenza virus, and morbidities and mortalities caused by influenza.

Influenza and Pregnancy

Our research presents a case to advocate for the implementation of influenza vaccination into prenatal care. Our data and literary research support the prediction that milk and colostrum contain important IgA and IgG antibodies for the prevention of infection and severe disease due to influenza. There is also clear literary evidence for the protection of newborns with maternal influenza vaccination. It is the best way to prevent adverse effects of influenza in both pregnant women and newborns up to six months old (Rasmussen, 2019). In newborns, adverse effects of influenza are indirectly prevented as preventing maternal infection reduces preterm birth, low birth weight, spontaneous

abortion, and birth defects (Azziz, 2021). For example, a study of 27,000 women found those who experienced fever due to influenza had greater instances of congenital abnormalities (Azziz, 2021). Directly, newborns are protected by the transfer of maternal antibodies until they can receive influenza vaccination at 6 months of life (Azziz, 2021). The immunosuppressed mother is also protected as the risk of hospitalization due to influenza is significantly higher for pregnant vs nonpregnant women (Azziz, 2021). Maternal vaccination is not associated with adverse prenatal/ neonatal outcomes, so the vaccine is safe and effective (Azziz, 2021). Data supports vaccination of mothers at any gestational age due to plentiful data supporting the safety and benefits; however, vaccination rates have decreased to about 50% (Azziz, 2021; Rasmussen, 2019). Obstetric practitioners must increase their efforts and strategies to improve vaccination rates to better protect newborns and mothers from adverse influenza outcomes (Rasmussen, 2019).

Universal Vaccination and the Pork Industry

A potential influenza pandemic can have devastating effects on the pork industry. The pandemic has direct negative effects as pigs are infected with the swine flu, causing financial loss due to reduced growth rates and acute respiratory disease (Pig, 2021). Along with the loss of livestock, the swine flu disrupts markets. For example, a few weeks after the first 2009 H1N1 outbreak, domestic pork demand decreased and 27 countries blocked United States imports causing the US pork industry to lose $1.1 billion in only six months (Pig, 2021).

The swine flu was transmitted through human-human or pig-human contact, and 15-25% of swine farmers had been exposed to the virus at work (Congressional, 2010).

Even though humans could not get the infection through properly handled pork products, negative perception and fear of swine caused financial loss. Domestic customers were leery about buying pork, with 13% of people believing they could contract the disease by eating pork (Congressional, 2010). This perception can be decreased by preventing interspecies transmission.

Universal vaccination for humans against a wide range of influenza strains prevents interspecies transmission. Prevention of interspecies and intraspecies transmission by universal vaccination would have an enormous impact on the pork industry in the face of a pandemic. Lower infection rates would decrease the negative perception of pork, preventing a decrease in pork product purchasing that occurred during the H1N1 pandemic. In addition, vaccinated pigs would be better protected, and producers would save money based on decreased morbidity and mortality of livestock.

CHAPTER FIVE

Conclusion

Results from the pig vaccine study indicate higher mean piglet titer values for PBS vaccinated vs HA-129 vaccinated mother pigs indicating increased protection in the HA-129 vaccination groups. Analysis reveals a p-value favorable of the hypothesis that if vaccinated mother pigs transfer high levels of antibodies to the newborn, then antibodies will protect influenza-challenged offspring from the virus. From the research, we can predict that IgA and IgG antibodies transferred to piglets for the HA-129 vaccinated group protected them from infection. Previous research of mice, chickens, and humans supports the maternal transfer of IgA and IgG, which are important for defense against influenza, through milk and colostrum. Further, research indicates passive protection of newborns with a 35% efficacy against the virus, supporting our hypothesis that piglets will be protected from adverse effects of influenza infection through passive maternal immunity (Azziz, 2021). With support from our results, our study aimed to increase advocacy for the vaccine with clear scientific research proving transferred protection to the newborn. Understanding whether using a universal vaccine delivered to mother pigs can transfer immunity to newborns is a step toward understanding whether a universal vaccine could create greater herd immunity against the influenza virus in humans, preventing deaths from the virus. The increased herd immunity would decrease influenza rates and aid the protection of vulnerable populations, such as those with immunodeficiency or the elderly. Furthermore, by studying whether this protects the vulnerable population of newborns, a basis for advocating the timing of influenza vaccination for pregnant women can be formed. Finally, because this vaccine is

universal, it would not need to be changed from year-to-year, so it could be implemented into the recommended prenatal care for pregnant women. Protection a universal influenza virus vaccine would provide to the general and vulnerable populations would decrease the annual large numbers of deaths and hospitalizations, the stress imposed on the healthcare system, and the negative economic impacts due to influenza.

Before the possibility of a universal vaccine, research focused on seasonal surveillance and vaccination against relevant influenza subtypes based on glycoproteins with high mutation rates (WHO, 2018). These HA (receptor binding) and NA (receptor destroying) glycoproteins allow pathogen attachment and release of virions (Hwang, 2020). After vaccination, antibodies and T-cells target these glycoprotein domains to prevent viral entry and release, reducing the spread of infection (Hwang, 2020). By preventing influenza infection through priming the immune response both seasonal and universal vaccines protect against secondary bacterial infection (Smith, 2018). This research shows that accurate protection against the influenza virus can be achieved by targeting conserved components of epitopes within universal vaccines. Our universal vaccine targets these conserved epitopes, such as the M2e protein which is present in all subtypes of influenza viruses, to prevent entry and infection of influenza (Hwang, 2020). These strategies have revealed protection against a broad spectrum of influenza subtypes decreasing the need for seasonal surveillance and annual vaccination if implemented. The aspect of broad-spectrum immunity and universal vaccines may be developed and improved for future broad immunity against influenza subtypes (Hwang, 2020).

This research can be expanded upon in the future. First, future research can be applied to definitively confirm the hypothesis that if vaccinated mother pigs transfer high

levels of antibodies to the newborn, then antibodies will protect influenza-challenged offspring from the virus. Additional research can be completed to determine the best timing for maternal vaccination against influenza to promote the optimal immune response in newborns. This research can be used to advocate for increased maternal vaccination in the clinical setting by setting a definitive timeline for recommended vaccination. Next, once a universal vaccine is approved for clinical use, research on herd immunity and the protection of vulnerable populations can be studied. This will provide a better source for protecting the elderly, infants, underprivileged groups, and immunocompromised individuals. Along with research into protection against influenza, the universal vaccine is being researched as a solution to COVID-19 to create a single vaccine targeting both respiratory diseases (Hwang, 2020). This bivalent vaccine would contain conserved SARS-CoV-2 and influenza antigens to provide a broad-spectrum immunity against subtypes of both groups, decreasing the viability of antigenic mutation as an effective way to evade the immune response (Hwang, 2020). Our research presents an important step to confirming the viability of the universal influenza vaccine and confirming the potential to expand universal vaccine research to emerging diseases in the future.

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